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Induction of Prophage Lambda by Chlorophenols

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16. ABSTRACT

Chlorinated phenols, which are used primarily as wood preservatives and fungicides, are present in most air, water, and soil samples in industrialized areas as well as in urine and body fat of most people. We have examined the ability of phenol and the 19 isomers of chlorophenol to induce DNA damage using the Microscreen prophage-induction assay in Escherichia coli. Seven of the isomers (2,3,4-tri, 2,4,5-tri, 3,4,5,-tri, 2,3,4,5-tetra, 2,3,6-tri, 2,4,6-tri, and pentachlorophenol) induced prophage lambda in the presence of S9, with the first three being 10 times more potent than the last three. The more potent isomers have either one or no chlorine atom ortho to the OH group; whereas the less potent isomers have two chlorine atoms ortho to the OH group. Although none of the 20 compounds is mutagenic in Salmonella, the prophage-induction results agree with findings by others that most of these seven isomers are clastogenic, are associated with cancer and chromosomal aberrations in humans, and are carcinogenic in rodents. A likely basis for the genotoxicity of the seven isomers involves the metabolism of the parent isomer to chlorohydroquinone, which can form a chlorobenzosemiquinone in the presence of oxygen. These two metabolites can produce free radicals that can cause DNA strand breaks, resulting in prophage induction in E. coli or the chromosomal aberrations/cancer associated with human exposure to chlorophenols.

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Induction of Prophage Lambda by Chlorophenols

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Chlorinated phenols, which are used primarily as wood preservatives and fungicides, are present in most air, water, and soil samples in industrialized areas as well as in the urine of most people. We have examined the ability of phenol and the 19 isomers of chlorophenol to induce DNA damage using the Microscreen prophage-induction assay in *Escherichia coli*. Seven of the isomers (2,3,4,-tri, 2,4,5-tri, 3,4,5-tri, 2,3,4,5-tetra, 2,3,6-tri, 2,4,6-tri, and pentachlorophenol) induced prophage lambda in the presence of S9, with the first three being ~10 times more potent than the last three. The more potent isomers have either one or no chlorine atom *ortho* to the OH group; whereas the less potent isomers have two chlorine atoms *ortho* to the OH group. Although none of the 20 compounds is mutagenic in *Salmonella*, the prophage-induction results agree with findings by others that most of these seven isomers are clastogenic, are associated with cancer and chromosomal aberrations in humans (pentachlorophenol), and are carcinogenic in rodents (2,4,6-tri and pentachlorophenol). A likely basis for the genotoxicity of the seven isomers involves the metabolism of the parent isomer to a chlorohydroquinone, which can form a chlorobenzosemiquinone in the presence of oxygen. These two metabolites can produce free radicals that can cause DNA strand breaks, resulting in prophage induction in *E. coli* or, possibly, the chromosomal aberrations/cancer associated with human exposure to chlorophenols.

Key words: Microscreen assay, prophage induction, DNA damage

INTRODUCTION

Chlorophenols are used widely as wood preservatives (fungicides), algicides, bacteriocides, heribicides, insecticides, and molluscicides [Cirelli, 1978; Rao, 1978; WHO, 1987]. Twenty years ago, the annual worldwide production of chlorophenols was estimated to be 200,000 metric tons [Paasivirta, 1978]. Global production of pentachlorophenol, which is the isomer used most extensively, has been estimated to be 90,000 metric tons [IRPTC, 1983], 80% of which is used for wood treatment [Crosby et al., 1981].

Human exposure to chlorophenols, especially pentachlorophenol, is considerable, with an estimated 3–6 million people being exposed annually in the United States to pentachlorophenol through occupational or household use [USDA, 1980]. Chlorophenols are also present in wood-preservative wastes [Donnelly et al., 1987a,b] and pulp and paper mill effluents [Nestmann et al., 1980; Nestmann and Lee, 1983], which, when discharged into rivers and streams, may introduce chlorophenols into drinking water. Consequently, the general population may be exposed to pentachlorophenol through drinking water (0.01–0.1 μg/liter) and food (up to 40 μg/kg in composite food samples) [WHO, 1987]. Considering the ubiquitous nature of these compounds, it is not surprising that ~85% of a random sample of residents of North America contained pentachlorophenol in their urine [Kutz et al., 1978].

In rodents, 2,4,6-trichlorophenol produced lymphomas and leukemias in male Fisher rats and hepatocellular carcinomas or adenomas in both male and female B6C3F$_1$ mice [NCI, 1979]. Pentachlorophenol produced hepatocellular carcinomas or adenomas in male and female B6C3F$_1$ mice [NTP, 1989a]. However, there was no evidence for the carcinogenicity of 2,4-dichlorophenol in male or female Fisher rats or B6C3F$_1$ mice [NTP, 1989b]. In humans, soft tissue sarcoma, malignant lymphoma, and leukemia have been associated with occupational exposure to 2,4,5-trichlorophenol, pentachlorophenol, and chlorophenol formulations containing mainly 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol (IARC, 1982). In addition, the lymphocytes of workers in a pentachlorophe-

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nol-producing factory showed a small but significant increase in chromosomal aberrations (dicentrics and acentrics) compared with control subjects [Bauchinger et al., 1982].

Although there is sufficient evidence for the carcinogenicity of several of the isomers of chlorophenol, these agents do not appear to induce point mutations. Neither phenol nor 17 isomers of chlorophenol were mutagenic in *Salmonella* [Haworth et al., 1983; Zeiger et al., 1988; NTP, unpublished results]; the two remaining isomers, 2,4-dichlorophenol and 3,5-dichlorophenol, produced questionable responses in *Salmonella* [Haworth et al., 1983]. However, 2,4,6-trichlorophenol was reported to be a potent inducer of DNA damage in the *Bacillus subtilis* rec-assay [Kinae et al., 1981].

In yeast in the absence of S9, 2,6-dichlorophenol and 2,4,5-trichlorophenol did not induce point mutations or mitotic gene conversion [Nestmann and Lee, 1983]; however 2,4,6-trichlorophenol induced forward mutation but not mitotic gene conversion, and pentachlorophenol induced both forward mutation and mitotic gene conversion [Fahrig et al., 1978]. Pentachlorophenol did not induce sex-linked recessive lethals in *Drosophila* [Vogel and Chandler, 1974]. In onion, 2,4-dichlorophenol and 2,4,5,-trichlorophenol induced sticky chromosomes and bridges, suggestive of chromosome breakage [Fiskesjo et al., 1981].

In mammalian cells, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol were not mutagenic at the *hprt* locus in V79 cells in the absence of S9 [Jansson and Jansson, 1986]. 2,4,6-Trichlorophenol did not induce chromosomal aberrations or sister chromatid exchanges (SCE) in CHO cells [Galloway et al., 1987], nor did 3,4,5-trichlorophenol (NTP, unpublished results). However, several other isomers, including 2,3,6-trichlorophenol, 2,3,4-trichlorophenol, 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, and pentachlorophenol, did induce chromosomal aberrations in CHO cells (NTP, unpublished results).

Although the data are limited, it appears that the chlorophenols may not induce point mutations. Instead, pentachlorophenol and some of the trichlorophenols and tetrachlorophenols induce chromosomal aberrations, are carcinogenic in rodents, and are associated with chromosomal aberrations and cancer in exposed people.

Because of the prevalence and persistence in the environment of carcinogenic chlorinated organics, as exemplified by the chlorophenols, there is a need for a short-term bioassay that can detect carcinogens in this chemical class. Although the *Salmonella* assay is the most widely used and convenient one for screening environmental samples [Waters et al., 1985], it has a low sensitivity for carcinogenic chlorinated organics (McCann et al., 1975; Zeiger and Tennant, 1986; Claxton et al., 1988]. In exploring the usefulness of the Microscreen prophage-induction assay in *Escherichia coli*, developed by Rossman et al. [1984], we have found that this assay detects more carcinogenic chlorinated organic pesticides and more hazardous wastes containing carcinogenic chlorinated organics than does the *Salmonella* assay [Houk and DeMarini, 1987, 1988]. Thus we have evaluated phenol and the 19 isomers of chlorophenol in the Microscreen prophage-induction assay to characterize further the genotoxicity of these agents. The results are discussed in terms of the probable mechanism by which certain isomeric classes of chlorophenol induce DNA damage, resulting in clastogenic and potential carcinogenic effects.

MATERIALS AND METHODS

Bacterial Strains

The lambda lysogen WP2$_s$(λ) (*lon*$_{11}$, *sul*A$_1$, *trpE*$_{65}$, *uvrA*$_{155}$, *lamB*$^+$) was derived from *E. coli* B/r and was obtained from Dr. Evelyn M. Witkin via Dr. Anne C. Frazer, Department of Microbiology, New York University Medical Center (New York, NY). The indicator strain TH-008 (Streptomycin^r) was derived from *E. coli* C and was obtained from Dr. A.C. Frazer. Single-colony isolates of each strain were grown overnight to stationary phase in nutrient broth (Oxoid No. 2). For phage induction, an aliquot of an overnight culture of WP2$_s$(λ) was diluted 1:40 in Vogel-Bonner minimal medium supplemented with 0.2% glucose and 20 μg/ml of tryptophan and grown to mid-log phase (~2 × 10^8 cells/ml). Cells were then centrifuged at 10,000 rpm for 10 min and resuspended in 10 times the original volume of fresh supplemented minimal medium to give a final concentration of ~2 × 10^7 cells/ml.

Chemicals

2-Nitrofluorene and 2-aminoanthracene were purchased from Sigma (St. Louis, MO). Phenol (Gold Label, Aldrich Chemical Co., Milwaukee, WI) was prepared by dissolving 500 g in 480 ml of chloroform (Fisher Scientific), 20 ml of isoamyl alcohol (Sigma), and 100 ml of Tris (50 mM, pH 7.4). The chlorophenols were obtained from the National Toxicology Program (National Institute of Environmental Health Sciences, Research Triangle Park, NC; their CASNR number and purity (%) are listed in Table I. Because phenol and 2-chlorophenol were liquids at room temperature, they were used directly in the assay and diluted in supplemented minimal medium. The remaining compounds were dissolved in acetone (Fisher Scientific) and diluted in medium.

Microsuspension Assay

Plastic, sterile 96-well microtiter plates (Corning Laboratory Sciences Co.) were used for the microsuspension

TABLE I. Chemicals Tested and Purity

Chemical	CASRN[a]	Purity (%)
Phenol	108-95-2	99
2-Chlorophenol (ortho)	95-57-8	Reagent grade
3-Chlorophenol (meta)	108-43-0	98
4-Chlorophenol (para)	106-48-9	Practical grade
2,3-Dichlorophenol	576-24-9	98
2,4-Dichlorophenol	120-82-2	99
2,5-Dichlorophenol	583-78-8	98
2,6-Dichlorophenol	87-65-0	99
3,4-Dichlorophenol	95-77-2	99
3,5-Dichlorophenol	591-35-5	99
2,3,4-Trichlorophenol	15950-66-0	97
2,3,5-Trichlorophenol	933-78-8	99
2,3,6-Trichlorophenol	933-75-5	99
2,4,5-Trichlorophenol	95-95-4	99
2,4,6-Trichlorophenol	88-06-2	Practical grade
3,4,5-Trichlorophenol	609-19-8	Unknown
2,3,4,5-Tetrachlorophenol	4901-51-3	98
2,3,4,6-Tetrachlorophenol	58-90-2	Unknown
2,3,5,6-Tetrachlorophenol	935-95-5	99
Pentachlorophenol	87-86-5	92

[a]Chemical Abstracts Service Registry Number.

assay. The first well in a dilution series received 250 μl of supplemented minimal medium and 50 μl of either the test compound or controls (acetone or medium). The remaining wells received 150 μl of the medium, and twofold serial dilutions of the compounds or controls (150 μl:150 μl) were made down the columns of each plate. Dilutions were extended into a second plate to assure identification of the dose range. Each well was inoculated with 75 μl ($\sim 2 \times 10^6$ cells) of the resuspended log-phase culture of WP2$_s$(λ) and 25 μl of medium or S9 mix (2.5%), prepared as described by Houk and DeMarini [1987] using rat liver homogenate (27.2 mg protein per ml of S9) from male Sprague-Dawley rats. The contents of each microtiter plate were mixed using a Minimixer (Fisher Scientific), the plates were covered with four layers of plastic wrap (Saran Wrap, Dow Chemical Co.), the lid was placed tightly on each plate, and the plates were incubated overnight at 37°C.

Assay for Plaque Formation

After incubation, the wells were scored for turbidity, with turbid wells indicating cell growth and clear wells indicating cytotoxicity and/or inhibition of cell growth. The concentration of lambda phage was determined by sampling at least the first five turbid wells adjacent to a clear well. Few or no phage are found in clear wells because of toxicity [Houk and DeMarini, 1987, 1988; Rossman et al., 1984, 1985]. A sample (50 μl) from the well was diluted in 5 ml of supplemented minimal medium, and the tube was vortexed. To ~2.5 ml of top agar (0.65% Bacto agar and 10 mM $MgSO_4$) were added 100 μl of the diluted phage and 200 μl of log-phase indicator cells (TH-008), which had been prepared by diluting an overnight culture of TH-008 1:40 in nutrient broth (Oxoid No. 2) and growing the culture until it reached a concentration of $\sim 2 \times 10^8$ cells/ml. The contents of the tubes were vortexed and poured onto bottom agar made of tryptone medium (10 g of Bacto tryptone, 5 g of NaCl, and 12 g of Bacto agar per liter of glass-distilled, deionized H_2O) supplemented with Streptomycin (100 μg/ml) to select against the lysogen. Plates were incubated overnight at 37°C, and plaques were counted by hand. The dilution tubes were sampled in duplicate, and all experiments were repeated at least twice.

Data Analysis

As observed previously [Houk and DeMarini, 1987], acetone (> 0.1%) induces prophage (Table II). Because of this dose-related response, the acetone (or medium) control plaque-forming units (PFU) per plate were subtracted from their corresponding test-compound values, giving the induced PFU/plate. In addition, the test-compound values were divided by their corresponding acetone controls to give the fold increase, which provided a further indication of the magnitude of induction [Houk and DeMarini, 1987, 1988].

The highest concentration of acetone at which any of the test compounds produced turbid wells that could be sampled for phage was 2.5%. Thus, as described previously [Houk and DeMarini, 1987, 1988], our criterion for a positive response for an experiment was a dose-related increase of induced PFU/plate that reached or exceeded the upper limit of the 99% confidence interval for 2.5% acetone. Based on the average PFU/plate for 2.5% acetone for the nine experiments reported here (Table II), the upper limit of the 99% confidence interval was 49 PFU/plate in the presence of S9 and 44 PFU/plate in the absence of S9. These values represent an approximate threefold increase over background. If a compound 1) reached or exceeded these values at only one dose or 2) reached or exceeded these values at more than one dose but did not produce a dose-related increase in PFU/plate, the result for that compound/experiment was scored as a weak positive (w^+). A summary response was given to each compound based on the reproducibility of the results from two independent experiments. If a given response was not reproducible, the summary response was scored as questionable (?).

RESULTS AND DISCUSSION

The induction of prophage lambda by phenol and the 19 isomers of chlorophenol is shown in Table III. All 20 compounds were tested to toxicity as indicated by clear wells. An additional indication of toxicity was exhibited by some of the compounds at doses that produced turbid wells as indicated by fold increases that were considerably less than one (e.g., 3-chlorophenol, Exp. 1, 75.8 μM, −S9). A fold

TABLE II. Induction of Prophage Lambda by Acetone

Actone (%)	PFU/plate ($\bar{X}$ ± S.E.M.)[a]	
	+S9	−S9
0.08	26 ± 2	22 ± 2
0.16	25 ± 2	22 ± 2
0.31	27 ± 2	26 ± 3
0.63	31 ± 2	25 ± 3
1.25	33 ± 6	28 ± 3
2.50	36 ± 4	32 ± 3
5.00	40 ± 6	34 ± 2
10.00	44 ± 6	35 ± 3

[a]Each mean was derived from 18 plates (nine independent experiments, each in duplicate).

increase of one indicates the induction of a background number of PFU/plate, i.e., a PFU/plate equal to that of the corresponding solvent control. As observed previously [Houk and DeMarini, 1987, 1988; Rossman et al., 1984, 1985], those doses that produced positive responses were generally those just below toxicity. The reason for this is most likely due to the biological consequences of prophage induction, i.e., cell lysis [Roberts and Devoret, 1983].

Of a total of 40 individual responses (two experiments for each of 20 compounds), reproducible summary responses (+, −, or w^+) were obtained for all but three pairs of responses, resulting in only three questionable (?) summary responses. Based on this qualitative assessment, the chlorophenols gave results in the assay that were generally highly reproducible. The quantitative reproducibility of the assay is discussed later.

In general, phenol, the monochlorophenols, and the dichlorophenols did not induce prophage lambda; however, three of the six dichlorophenols produced w^+ summary responses in the presence of S9 (Table III). Although the isomers containing only one or two chlorine atoms were negative, positive summary responses were produced by all but one of the trichlorophenols, 2,3,4,5-tetrachlorophenol, and pentachlorophenol in the presence of S9 (Table III). Four of these isomers, 2,3,5-trichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol, also gave w^+ summary responses in the absence of S9 (Table III). 2,3,5,6-Tetrachlorophenol produced a w^+ summary response in the presence of S9. Thus the more highly chlorinated isomers were more likely to be genotoxic as evidenced by the finding that the monochlorophenols and dichlorophenols were generally not genotoxic, whereas pentachlorophenol and most of the trichlorophenols and tetrachlorophenols were genotoxic. In addition, positive responses occurred only in the presence of S9 (Table III).

A general definition of potency is the concentration of an agent that is required to produce a particular response. As described in Materials and Methods, we used the linear portion of the dose-response curve of each compound that gave a positive response in order to calculate the concentration (μM) required by that compound to produce an induced PFU/plate of 49 (+S9) and 44 (−S9), which represented the upper limit of the 99% confidence interval for acetone at a concentration of 2.5% (Table IV). As observed previously [Houk and DeMarini, 1987, 1988], these values corresponded to an approximate threefold increase over background, which was the same fold increase recommended by Rossman et al. [1985] for a positive response.

Each of the potency values represents the minimum concentration required to produce a positive response, i.e., the lowest effective concentration (LEC). Based on these LEC values, the compounds were ranked from most to least potent (Table IV). The relative potencies of the seven positive chlorophenols (all in the presence of S9) are illustrated in Figure 1, which shows the dose-response curve for the experiment with the highest correlation coefficient (r^2 value) for each compound. Three of the trichlorophenols (2,3,4-tri, 3,4,5-tri, and 2,4,5-tri) were similar in potency and were ~10 times more potent than the other three isomers whose potencies also clustered (penta, 2,4,6-tri, and 2,3,6-tri). 2,3,4,5-Tetrachlorophenol was intermediate in potency to these six isomers. These quantitative results revealed that of the seven genotoxic isomers, those that contained either one or no chlorine atoms *ortho* to the OH group were approximately one order of magnitude more potent than those that contained two chlorine atoms *ortho* to the OH group (Table IV, Fig. 1).

These structure–activity relationships and the requirement for S9 by the seven genotoxic isomers may be explainable based on the observations of Witte et al. [1985] for pentachlorophenol. The authors showed that pentachlorophanol was metabolized by rat liver S9 to tetrachlorohydroquinone. Although pentachlorophenol itself did not bind covalently to calf thymus DNA or cause single-strand breaks in bacteriophage PM2 DNA, tetrachlorohydroquinone did bind covalently to calf thymus DNA and induced single-strand breaks in PM2 DNA [Witte et al., 1985]. In addition, the authors showed that the cytotoxicity of tetrachlorohydroquinone to human fibroblasts was greater than that of pentachlorophenol (+S9), which was greater than that of pentachlorophenol (−S9). Hydroquinones can form semiquinone radicals in the presence of oxygen, and Witte et al. [1985] also detected the tetrachloro-*p*-benzosemiquinone radical in the pentachlorophenol/S9 incubation.

As reviewed in the Introduction, none of the 19 isomers of chlorophenol is mutagenic in *Salmonella* nor do they appear to induce point mutations in any other test systems. However, pentachlorophenol and some of the trichlorophenols and tetrachlorophenols are clastogenic. The standard *Salmonella* strains do not revert well by strand breaks caused by free radicals [Levin et al., 1982]; however, single-strand breaks are one of the most potent inducers of prophage lambda (or the SOS response in general)

TABLE III. Induction of Prophage Lambda by Phenol and Chlorophenols

Phenol or chlorophenol	Dose (μM)	Induced PFU/plate (fold increase)[a] Experiment 1 +S9	Experiment 1 −S9	Experiment 2 +S9	Experiment 2 −S9	Summary response +S9	Summary response −S9
Phenol	1,110.00	0 (0.9)	0 (0.9)	17 (2.4)	11 (1.7)		
	2,220.00	0 (0.9)	0 (0.9)	9 (1.3)	8 (1.5)		
	4,440.00	0 (0.6)	0 (0.3)	0 (0.6)	12 (1.9)		
	8,880.00	Toxic	Toxic	0 (0.6)	Toxic		
	17,760.00			Toxic			
Response		—	—	—	—	—	—
2-Mono	121.00			0 (0.5)	0 (1.3)		
	242.00	0 (0.2)	0 (0.1)	0 (0.8)	0 (0.2)		
	483.00	0 (0.1)	0 (0.2)	0 (0.2)	0 (0.1)		
	967.00	0 (0.2)	0 (0.4)	0 (0.1)	Toxic		
	1,933.00	6 (1.2)	0 (0.1)	0 (0.1)			
	3,867.00	17 (1.5)	0 (0.2)	Toxic			
	7,734.00	Toxic	Toxic				
Response		—	—	—	—	—	—
3-Mono	37.90	34 (1.9)	26 (2.5)	18 (2.1)	42 (3.4)		
	75.80	19 (1.5)	0 (0.3)	15 (2.2)	42 (4.0)		
	152.00	15 (1.5)	0 (0.5)	9 (1.3)	20 (2.3)		
	303.00	0 (0.7)	Toxic	11 (1.4)	Toxic		
	606.00	Toxic		62 (3.9)			
	1,212.00			Toxic			
Response		—	—	w[+]	—	—	?
4-Mono	75.80	18 (1.5)	0 (0.5)	22 (2.8)	20 (2.4)		
	152.00	29 (2.0)	0 (0.3)	0 (0.7)	32 (3.1)		
	303.00	29 (1.8)	0 (0.1)	0 (0.9)	30 (3.3)		
	606.00	0 (0.7)	Toxic	2 (1.0)	Toxic		
	1,212.00	Toxic		15 (1.9)			
	2,424.00			Toxic			
Response		—	—	—	—	—	—
2,3-Di	19.28	49 (2.7)	10 (1.3)	39 (2.3)	16 (1.5)		
	38.56	23 (1.6)	38 (2.0)	11 (1.4)	24 (1.8)		
	77.12	27 (1.6)	7 (1.2)	74 (3.3)	2 (1.0)		
	154.24	Toxic	Toxic	29 (1.8)	Toxic		
	308.48			Toxic			
Response		w[+]	—	w[+]	—	w[+]	—
2,4-Di	7.50	23 (2.0)	0 (0.4)				
	15.00	3 (1.1)	0 (0.0)	10 (1.3)	27 (2.5)		
	30.00	0 (0.2)	0 (0.1)	0 (0.8)	7 (1.4)		
	60.00	Toxic	Toxic	10 (1.2)	12 (1.3)		
	120.00			0 (0.9)	0 (0.8)		
	240.00			6 (1.1)	16 (1.5)		
	480.00			Toxic	Toxic		
Response		—	—	—	—	—	—
2,5-Di	2.41	0 (0.8)	2 (1.0)	23 (1.7)	1 (1.0)		
	4.82	26 (1.9)	8 (1.2)	34 (2.2)	15 (1.4)		
	9.64	11 (1.3)	7 (1.2)	34 (2.3)	31 (2.0)		
	19.28	50 (2.7)	Toxic	45 (2.5)	31 (2.0)		
	38.56	45 (2.2)		103 (4.9)	Toxic		
	77.12	Toxic		Toxic			
Response		w[+]	—	w[+]	—	w[+]	—
2,6-Di	19.28	38 (2.3)	5 (1.1)	19 (1.6)	19 (1.6)		
	38.56	19 (1.5)	12 (1.3)	29 (2.1)	20 (1.6)		
	77.12	35 (1.8)	17 (1.4)	82 (3.6)	0 (1.0)		
	154.24	49 (2.0)	0 (0.0)	8 (1.2)	Toxic		
	308.48	Toxic	Toxic	Toxic			
Response		w[+]	—	w[+]	—	w[+]	—
3,4-Di	9.64	13 (1.4)	10 (1.3)	26 (2.0)	5 (1.1)		
	19.28	26 (1.9)	5 (1.1)	20 (1.6)	2 (1.0)		
	38.56	15 (1.4)	8 (1.2)	32 (2.2)	0 (1.0)		
	77.12	10 (1.2)	0 (0.1)	27 (1.8)	Toxic		
	154.24	Toxic	Toxic	Toxic			

(continued)

Table III (*continued*)

Response		—	—	—	—	—	—
3,5-Di	4.82	30 (2.0)	5 (1.1)	24 (1.8)	0 (0.8)		
	9.64	18 (1.5)	0 (1.0)	26 (2.0)	0 (1.0)		
	19.28	38 (2.3)	4 (1.1)	40 (2.3)	0 (0.8)		
	38.56	54 (2.5)	Toxic	52 (3.0)	Toxic		
	77.12	0 (0.8)		Toxic			
	154.24	Toxic					
Response		w+	—	w+	—	w+	—
2,3,4-Tri	1.99	89 (2.3)	13 (1.2)	54 (3.0)	0 (0.9)		
	3.99	199 (6.3)	88 (4.3)	62 (3.5)	60 (3.8)		
	7.97	395 (12.9)	61 (3.4)	255 (11.2)	67 (3.5)		
	15.94	401 (11.0)	37 (1.7)	257 (11.7)	16 (1.6)		
	31.88	150 (4.2)	0 (0.9)	120 (5.6)	4 (1.1)		
	63.75	Toxic	Toxic	Toxic	Toxic		
Response		+	w+	+	w+	+	w+
2,3,5-Tri	3.99	46 (2.6)	0 (0.9)	28 (2.0)	0 (0.9)		
	7.97	48 (2.4)	0 (0.7)	60 (3.3)	1 (1.0)		
	15.94	44 (2.5)	0 (0.6)	29 (1.9)	0 (0.4)		
	31.88	32 (1.9)	Toxic	31 (2.1)	Toxic		
	63.75	27 (1.6)		15 (1.4)			
	127.50	Toxic		Toxic			
Response		—	—	w+	—	?	—
2,3,6-Tri	15.94	30 (2.7)	10 (1.6)	63 (4.0)	5 (1.2)		
	31.88	34 (2.3)	10 (1.6)	53 (3.1)	0 (0.7)		
	63.75	95 (4.5)	17 (2.0)	118 (8.8)	0 (0.7)		
	127.50	Toxic	Toxic	101 (5.5)	Toxic		
	255.00			Toxic			
Response		+	—	+	—	+	—
2,4,5-Tri	3.99	63 (3.6)	43 (3.0)	80 (3.1)	80 (4.0)		
	7.97	133 (6.3)	18 (1.6)	244 (8.3)	49 (2.9)		
	15.94	208 (9.6)	6 (1.2)	341 (9.5)	0 (0.8)		
	31.88	237 (10.1)	Toxic	257 (6.5)	Toxic		
	63.75	Toxic		Toxic			
Response		+	w+	+	w+	+	w+
2,4,6-Tri	7.97	31 (2.2)	46 (2.7)	74 (3.2)	52 (3.0)		
	15.94	43 (2.7)	61 (3.3)	46 (2.1)	18 (1.3)		
	31.88	97 (4.7)	30 (2.3)	139 (4.0)	40 (1.9)		
	63.75	100 (4.1)	0 (0.6)	195 (3.5)	2 (1.0)		
	127.50	35 (1.8)	Toxic	100 (2.6)	Toxic		
	255.00	Toxic		Toxic			
Response		+	w+	+	w+	+	w+
3,4,5-Tri	1.99	61 (1.9)	17 (1.3)	69 (3.6)	16 (1.7)		
	3.99	100 (3.7)	33 (2.2)	84 (4.5)	27 (2.0)		
	7.97	226 (7.8)	30 (2.2)	238 (10.5)	31 (2.1)		
	15.94	178 (5.4)	Toxic	248 (11.3)	Toxic		
	31.88	Toxic		Toxic			
Response		+	—	+	—	+	—
2,3,4,5-Tetra	0.85	30 (2.3)	0 (0.4)	5 (1.2)	12 (1.8)		
	1.70	37 (2.1)	0 (0.7)	11 (1.4)	9 (1.3)		
	3.40	46 (3.7)	35 (2.8)	20 (2.1)	2 (1.1)		
	6.79	74 (4.8)	Toxic	103 (5.7)	Toxic		
	13.59	342 (17.2)		157 (9.7)			
	27.17	Toxic		Toxic			
Response		+	—	+	—	+	—
2,3,4,6-Tetra	5.30	29 (2.2)	0 (0.1)				
	10.60	0 (0.8)	0 (0.1)	31 (2.1)	23 (2.2)		
	21.19	0 (0.2)	0 (0.1)	11 (1.3)	31 (2.8)		
	42.39	Toxic	Toxic	17 (1.4)	10 (1.3)		
	84.77			8 (1.2)	0 (0.1)		
	169.54			0 (0.9)	0 (0.1)		
	339.08			Toxic	Toxic		
Response		—	—	—	—	—	—
2,3,5,6-Tetra	13.59	12 (1.6)	49 (4.2)	35 (2.6)	0 (0.8)		
	27.17	28 (2.1)	44 (3.7)	33 (2.3)	7 (1.4)		
	54.34	18 (2.8)	32 (2.8)	12 (1.8)	3 (1.1)		

(*continued*)

Table III (*continued*)

	108.68	132 (5.4)	Toxic	116 (6.2)	Toxic		
	217.36	Toxic		Toxic			
Response		w⁺	w⁺	w⁺	—	w⁺	?
Penta	1.47	4 (1.1)	0 (0.8)	26 (1.8)	4 (1.1)		
	2.94	16 (1.8)	49 (3.7)	41 (3.4)	5 (1.2)		
	5.87	7 (1.3)	58 (5.5)	20 (2.0)	3 (1.1)		
	11.75	53 (3.9)	42 (3.8)	29 (2.3)	0 (1.0)		
	23.50	105 (5.0)	107 (7.7)	87 (4.4)	36 (3.1)		
	46.99	175 (7.5)	Toxic	151 (11.0)	Toxic		
	93.98	Toxic		Toxic			
Response		+	w⁺	+	w⁺	+	w⁺

[a]Induced PFU/plate is the observed minus the background; each value is the average of two plates. The positive control results for 2-aminoanthracene (3.24 μM) in the presence of S9 were 854 induced PFU/plate, 24.7-fold increase (mean of nine experiments). The positive control results for 2-nitrofluorene (710.90 μM) in the absence of S9 were 887 induced PFU/plate, 27.9-fold increase (mean of seven experiments).

[Elespuru, 1984]. In addition, oxidative damage to DNA generally results in clastogenic events (strand breaks/chromosomal aberrations) rather than gene or point mutations [Hsie et al., 1986; Evans et al., 1987].

Thus a likely mechanism by which the penta, tetra, and tri isomers of chlorophenol are genotoxic may involve the recognition of these isomers (and no or little recognition of the mono and di isomers) by enzymes that metabolize these isomers to chlorohydroquinones, which may then form chlorobenzosemiquinone radicals. These radicals may produce oxidative damage to DNA that results primarily in strand breaks, leading to the induction of prophage lambda in *E. coli* or to chromosomal aberrations in mammalian cells. The recognition of the enzymes for certain isomers, as well as the ability of a particular isomer to form the semiquinone radical, may be a function of the distribution of chlorine atoms on the phenol molecule, which may be the basis for the different genotoxic potencies of the isomers. This is also suggested by the fact that there is a rough, inverse relationship between the magnitude of the pKa for each of the 19 isomers and the genotoxicity of each isomer (data not shown).

Consistent with the results reported here are the findings that hydroquinone itself was not mutagenic in *Salmonella* [Haworth et al., 1983], but it was clastogenic in CHO cells [Galloway et al., 1987]. Of the seven isomers that induced prophage, 2,3,6-trichlorophenol, 2,3,4-trichlorophenol, 2,3,5,6-tetrachlorophenol, and pentachlorophenol were also clastogenic in CHO cells (NTP, unpublished results). When wood-preserving wastes were fractionated and studied for genotoxicity, the acid fraction, which contained pentachlorophenol, was the only fraction that induced DNA damage in the *B. subtilis* rec-assay [Donnelly et al., 1987b], and it was the most clastogenic fraction when evaluated for the induction of major chromosomal abnormalities in *Aspergillus nidulans* [Donnelly et al., 1987a].

Of the three isomers that have been tested for carcinogenicity, 2,4-dichlorophenol was neither carcinogenic [NTP, 1989b] nor induced prophage, whereas both 2,4,6-trichlorophenol and pentachlorophenol were carcinogenic [NCI, 1979; NTP, 1989a] and induced prophage. In addition, workers exposed to pentachlorophenol had elevated levels of chromosomal aberrations [Bauchinger et al., 1982], and people exposed to pentachlorophenol and some of the tetrachlorophenols and trichlorophenols (most of which induced prophage) had higher incidences of soft tissue sarcoma, malignant lymphoma, and leukemia than did control populations [IARC, 1982]. Thus the potential clastogenic effects of pentachlorophenol and most of the tetrachlorophenols and trichlorophenols may be the basis for their carcinogenic activity.

Considering the genotoxic/carcinogenic effects of some isomers of chlorophenol, as well as their presence in air, soil, water, and people [Rao, 1978; WHO, 1987], efforts should be made to minimize human exposure to these compounds. In this regard, efforts to employ various microbes to dechlorinate pentachlorophenol in the environment should proceed with caution because some microbes degrade pentachlorophenol to some of the trichlorophenols [Reineke and Knackmuss, 1988], which are even more genotoxic than the parent compound (Table IV, Fig. 1).

Although the data base is limited, the present results with chlorophenols, along with those for chlorinated pesticides [Houk and DeMarini, 1987] and hazardous wastes containing carcinogenic chlorinated organics [Houk and DeMarini, 1988], suggest that prophage induction may be able to detect clastogenic-type events produced by certain chlorinated organics that would otherwise be missed by the standard *Salmonella* assay. Because of the prevalence of carcinogenic chlorinated organics in the environment, there is a need for an assay to complement the *Salmonella* assay for the detection of this chemical class of environmental pollutant. Prophage induction in *E. coli* may be a candidate assay for this purpose.

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TABLE IV. Relative Potencies of Chlorophenols in the Presence of S9

Chlorophenol	Experiment 1			Experiment 2		
	r^2	LEC[a]	Rank	r^2	LEC[a]	Rank
2,3,4-Tri	0.99	1.14	1	0.96	2.56	3
3,4,5-Tri	0.99	1.81	2	0.97	1.92	2
2,4,5-Tri	0.99	2.01	3	0.94	0.86	1
2,3,4,5-Tetra	0.97	4.47	4	0.95	4.40	4
Penta	0.99	9.14	5	0.99	16.28	7
2,4,6-Tri	0.99	15.79	6	0.95	10.61	5
2,3,6-Tri	0.96	34.41	7	0.89	14.49	6

[a]LEC is the lowest effective concentration (μM) required to produce an induced PFU/plate (+ S9) at the upper limit of the 99% confidence interval based on the acetone (2.5%) controls. This is the concentration required to produce 49 induced PFU/plate in the presence of S9.

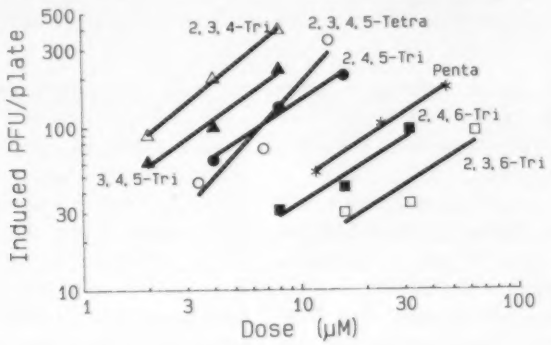

Fig. 1. Dose-response curves of prophage induction in the presence of S9 by the seven chlorophenols that gave reproducible (+) results. Data are from Table III, experiment 1. The r^2 and LEC values are given in Table IV.

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